Residues of organochlorine pesticides and polycyclic aromatic hydrocarbons in farm-raised livestock feeds and manures in Jiangsu, China

Ling Zhao, Yuan-Hua Dong *, Hui Wang

Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China
Graduate University of Chinese Academy of Sciences, Beijing 100049, China

HIGHLIGHTS

► Slightly higher mean residuals of OCPs were found in manures than in feeds.
► α-HCH was the most abundant compound in all kinds of animal feeds and manures.
► The predominance of p,p'-DDE and p,p'-DDT of total DDTs was clearly observed.
► Phenanthrene was the most dominant PAH species in each kind of animal manure.
► PAHs with 3 rings were the primary components in the tested manures.

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ABSTRACT

The residual levels of 8 organochlorine pesticides (OCPs) and 15 priority polycyclic aromatic hydrocarbons (PAHs) were determined in pig, chicken, and cow feed and manure samples collected from feedlots in Jiangsu province, China. The mean residuals of OCPs ranged from 25.35 to 65.62 ng g⁻¹ in feeds and from 33.46 to 90.89 ng g⁻¹ in manures. Among 4 hexachlorocyclohexanes (HCHs), α-HCH was the most abundant compound, with a high occurrence above 80% in all kinds of animal feeds and manures. For dichlorodiphenyltrichloroethanes (DDTs), the predominance of p,p'-DDE and p,p'-DDT of total DDTs was also clearly observed. Composite profiles of HCHs and DDTs in feeds indicated that the residuals of lindane and DDTs could be attributed to new inputs in the past several years. The mean residuals of all of the PAHs varied from 128.94 to 389.66 ng g⁻¹ in manures. The mean concentrations of seven carcinogenic PAHs in manures varied from 16.80 to 79.70 ng g⁻¹. Of the 15 priority PAHs, phenanthrene was the most dominant PAH species and accounted for approximately 50% of the total PAHs in all animal manures. The distribution of PAHs with different rings showed that PAHs with 3 rings were the primary components in the tested manures.

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1. Introduction

Hexachlorocyclohexane isomers (HCHs, including α-HCH, β-HCH, γ-HCH and δ-HCH) and dichlorodiphenyltrichloroethane and its metabolites (DDTs, o,p'-DDT, p,p'-DDT, DDD and DDE), the important components of organochlorine pesticides (OCPs), were extensively used in agriculture and vector control in China from the 1950s to the 1980s. During this period, the production of HCHs and DDTs was about 4.9 and 0.4 million tons, respectively, accounting for 33 and 20% of the total world production (Zhang et al., 2002). Although the application of OCPs has been banned from agricultural use since the early 1980s, for industrial HCHs, and early 1990s for DDTs and lindane (almost pure γ-HCH) (Chen, 1990), 3200 t of lindane continued in use until 2000 for the control of forest pests. DDT was also produced for export and/or for further production of dicofol, another pesticide (Li et al., 1999, 2001). After the ban on OCPs usage in 1983, residuals of HCHs and DDTs in various environmental media have declined considerably, but large amounts still remain in the environment due to high persistency, possible illegal use, or occurrence of DDTs as an impurity in widely applied dicofol (Chen et al., 2005a; Wu et al., 2005).

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants formed from the combustion of fossil fuels and are always found as a mixture of individual compounds (Wilson and Joins, 1993). Sixteen PAHs have been identified as priority pollutants by both China and the United States Environmental Protection Agency and seven of them are considered as probably carcinogenic (Menzie and Potokib, 1992). The determination and monitoring of PAHs in environmental samples and foodstuffs are necessary and important to human health (García-Falcón et al., 2004, 2006; Rey-Salgueiro et al., 2008a,b; Rey-Salgueiro et al., 2009).
Because of high persistence, bioaccumulation and toxicity of OCPs and PAHs, pollution by them is still of wide concern. In recent years, a larger number of studies have reported OCPs and PAHs contamination in China; however, most studies examined the residuals of these pollutants in soils, foodstuffs, vegetables, organisms, water and sediments (Tao et al., 2004,2009; Zhang et al., 2004; Chen et al., 2005b; Song et al., 2006; Hu et al., 2009). On the other hand, commercial livestock production using similar facilities and commercial feeds has increased rapidly in recent years in China, and concomitantly large numbers of animal manures are produced during large-scale animal feeding operations. Animal manure is not only a source of valuable plant nutrients, but can also be a source of air pollution and a threat to aquifers and surface water because animal manure contains trace nutrient elements, viruses, heavy metals, veterinary antibiotics and other pollutants (Sager, 2007; Venglovsky et al., 2009). In addition, the animal manure can be used as feeds for the fish to reduce the requirement for expensive feeds and fertilizers (Salazar and Saldana, 2007). The residual of heavy metals and veterinary antibiotics have been well documented (Salazar and Saldana, 2007; Zhao et al., 2010; Pan et al., 2011); however, little attention has been paid to OCP and PAH residuals in feed and manure samples from farm-raised animals in China. Therefore, screening for persistent organic pollutants, such as OCPs and PAHs in farm-raised animal feeds and manures is imperative for food safety and environmental protection. In this study, the objective was to investigate OCPs and PAHs residual levels in animal feeds and manures at different livestock and poultry farms in Jiangsu province of China and evaluate a possible influence of the residual of OCPs and PAHs on the environment.

2. Materials and methods

2.1. Chemicals and methods

High-performance liquid chromatographic (HPLC) grade n-hexane, petroleum ether, dichloromethane, acetone, acetonitrile and methanol were purchased from Tedia Company Inc. (Fairfield, OH). Ultra pure water was provided with a Milli-Q Advantage A10 Water Purification System (US Millipore Co., Bedford, MA). All glassware was pre-washed and rinsed with distilled methanol before use. Anhydrous sodium sulfate was dried at 450 °C for 6 h and stored in a sealed desiccator. The reference standards mixture of eight OCPs (α-HCH, β-HCH, γ-HCH, δ-HCH, o,p′-DDT, p,p′-DDT, p,p′-DDD and p,p′-DDE) at a concentration of 100 mg l⁻¹ were purchased from the National Research Center for Certified Reference Materials, China. The standard mixtures of OCPs were diluted with petroleum ether for calibration standards in the range of 1.0–200.0 μg l⁻¹. Standard PAHs (16 compounds specified in EPA Method 610) in a mixture were obtained from Supelco (Bellefonte, PA). These compounds are as follows: naphthalene (Nap), acenaphthylene (Ac), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phen), anthracene (Anth), fluoranthene (Flt), pyrene (Pyr), chrysene (Chry), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3-cd]pyrene (Inpy), benzo[gh, ij]perylene (BghiP) and dibenzo[a,h]anthracene (DahA). Working standards of PAHs were prepared by diluting the standard mixture with acetonitrile in the range of 0.01–10 mg l⁻¹.

2.2. Sample collection

Samples were collected from 12 feedlots that are located in Lianyungang, Huai'an, Taizhou, Yangzhou, Nanjing, Dongtai, Haian, Rugao, Changzhou, Liyang, Wuxi and Suzhou, from the North to the South in Jiangsu province, in June, 2005. Eight pig feed samples and 15 pig feces samples were collected from 10 pig farms (70–5000 pigs in total at a farm). Eight feed samples and 12 manure samples were collected from 11 chicken farms (1200–1,000,000 chickens in total at a farm). Six feed samples and 8 manure samples were collected from 7 cow farms (100–700 cows in total at a farm).

Each manure sample was placed into a plastic container. All samples were air-dried at room temperature away from light after being taken to the laboratory. The samples were ground and sieved (60 mesh), and stored in closed vessels for further analysis.

2.3. Sample extraction, clean-up and analysis

2.3.1. OCPs

An aliquot (10 g) of feed or manure sample was weighed accurately and mixed with 10 g of copper powder (100 mesh) that was used as a desulfurating agent. The mixtures were extracted by Soxhlet-extraction in a 60 °C water-bath for 6 h with 60 ml 1:1 petroleum ether/acetone (V/V). The extracts were transferred to a 250 ml separatory funnel and sulfonated with 10 ml of concentrated sulfuric acid several times until the sulfuric acid layer became colorless. After the sulfuric acid layer was discarded, the petroleum ether layer was purged with 6% sodium sulfate solution until the pH value of the extraction system was greater than 5.0. Finally, the petroleum ether layer was dehydrated with anhydrous sodium sulfate and concentrated by a rotary evaporator to 25 ml for GC detection.

The concentrated extracts were analyzed by an Agilent 6890 N GC, coupled with a micro-electron capture detection (μ-ECD) system, 7683 auto-injector and chromatographic working station. The GC column was a DB-1701 fused silica capillary column (J&W Scientific Inc.) that was 0.32 mm × 30 m × 0.25 μm. The injection conditions were: injector temperature, 210 °C; μ-ECD temperature, 320 °C; and the oven temperature started at 165 °C (holding time 2 min) and then increased to 265 °C at 6 °C min⁻¹ (holding time 2 min). The carrier gas was helium with a flow rate of 2 ml min⁻¹, and the make-up gas was nitrogen with a flow rate of 60 ml min⁻¹. The injection volume was 1.0 μl, which was injected into a split/splitless inlet operated in the splitless mode. The calibration method was based on a six-point calibration curve for individual components (1.0–200.0 ng ml⁻¹). Linear fits were used for all analyses, and correlation coefficients were > 0.99.

2.3.2. PAHs

Only four pig, two chicken and two cow manure samples collected from Nanjing farms were selected to determine the residuals of PAHs in manure samples. To extract PAHs from the manure samples, 10 g of dry sample were placed in Soxhlet-extraction thimbles and extracted for 24 h with 60 ml of dichloromethane: acetone (1:1) in a 60 °C water-bath. The extracts were then reduced to near dryness on a rotary evaporator. The extracts were redissolved in about 2 ml of cyclohexane and cleaned by a chromatography column filled with 10 mm of anhydrous sodium sulfate, 80 mm of silica gel, 60 mm of neutral alumina and 10 mm of anhydrous sodium sulfate. For the cleaning, 15-ml of hexane:dichloromethane (1:1) was added to a 60 °C water-bath. The extracts were then reduced to near dryness on a rotary evaporator. The extracts were redissolved in about 2 ml of cyclohexane and cleaned by a chromatography column filled with 10 mm of anhydrous sodium sulfate, 80 mm of silica gel, 60 mm of neutral alumina and 10 mm of anhydrous sodium sulfate. For the cleaning, 15-ml of hexane:dichloromethane (1:1) was added for elution at the rate of 1 ml min⁻¹ and the 3-ml beginning of the elution fraction was discarded, then the remaining elution fraction was collected and concentrated to near dryness by a rotary evaporator and redissolved in 1 ml of acetonitrile for HPLC analysis.

Determinations of PAHs were performed using a 2695 Waters Alliance system (Milford, MA, USA) equipped with an autosampler-controlled binary gradient system. A Waters® PAH C18 column (4.6 × 250 mm, particle size 5 μm; Waters Corporation, Milford, MA) with a Waters C18 guard cartridge (4.0 × 3.0 mm) used to separate PAHs. A Waters 2475 fluorescence detector was used to measure PAHs. The native fluorescence of the PAHs was used for their detection and quantification, and PAHs were determined by their retention times calibrated by a mixed standard sample from Supelco Company containing 16 individual PAHs. Acenaphthylene was not included in this study because of its low fluorescence properties. The chromatographic method employed an injection volume of 10 μl at 30 °C column temperature and...
a flow rate of 1.0 ml min\(^{-1}\). The mobile phase consisted of water (solvent A) and acetonitrile (solvent B). Elution started with 60% B for 11 min and was followed by a 14-min linear gradient to 99% B. After a 25-min elution described above, the initial conditions were reestablished and held for 20 min. The detection conditions, including the FLD operating parameters and the retention times of 15 individual PAHs, and the composition of the PAHs standard mixtures are given in Table 1.

### 2.4. Quality control and quality assurance (QA/QC)

The blank and duplicate samples were added in the analysis of each sample (10 feed and 10 manure samples). The limits of detection (LOD) and quantification (LOQ) for the instrument were determined using the standard deviation of the response and the slope of the calibration curves containing seven concentrations for OCPs or PAHs (ICH Steering Committee, 1996). The method quantification limit (MQL) determination in this study was based on the United States Environmental Protection Agency method that used the variability of multiple analyses of extracts obtained from residue-free pig manure spiked at 8 ng g\(^{-1}\) for OCPs and at 400 ng g\(^{-1}\) for PAHs (Berthouex and Brown, 2002). Results for LOD, LOQ, recovery rate and MQL of the selected OCPs and PAHs are summarized in Table 2. The MQLs of 23 analytes were 1.94–4.72 ng g\(^{-1}\) for OCPs and 1.32–74.02 ng g\(^{-1}\) for PAHs. In the recovery experiments, the overall mean recoveries for all target analytes in the spiked pig manure samples ranged from 42.8 to 105.3%, with a RSD lower than 10%, confirming the validity of the methodology applied in this work. All the residue levels in this study were expressed on dry weight.

### 3. Results and discussion

#### 3.1. OCP residuals

##### 3.1.1. Levels of OCP residuals in animal feeds

The measured residuals of HCHs and DDTs in various animal feed samples are listed in Table 3. For approximately 50% of the feed samples, two or more HCH and DDT isomers could be determined in a single pig, chicken, or cow feed sample. The total residuals of the means from the individual HCHs were 7.36, 7.39, and 6.55 ng g\(^{-1}\) in pig, chicken, and cow feeds, respectively. Among the 4 HCHs, α-HCH was observed in all of the chicken and cow feeds, followed by β-HCH with an occurrence of 62.5% and 75% in pig and chicken feeds, respectively. Likewise, the mean concentrations of α-HCH were the highest in all feeds for the three animals, whereas only a relatively high mean residual level of 2.69 ng g\(^{-1}\) for β-HCH was found in chicken feeds. γ-HCH was detected in half of the cow feeds, and the mean concentrations of γ-HCH were 1.89, 0.74, and 4.59 ng g\(^{-1}\) in pig, chicken, and cow feeds, respectively. Both the occurrence and residue level of α-HCH were the lowest in the three kinds of animal feed samples, especially in cow feeds. The total residuals of the means from the individual DDTs were 10.38, 58.23, and 19.42 ng g\(^{-1}\) in pig, chicken, and cow feeds, respectively. On the whole, the DDT residuals were slightly higher than those of the HCH residuals in each kind of animal feed. Among the 4 DDTs, p,p′-DDE showed the highest occurrence in each kind of animal feed, followed by p,p′-DDE. The occurrence of p,p′-DDE and p,p′-DDD was very low in both pig and chicken feeds, and these two DDT isomers were not detectable in cow feeds, exhibiting a significant difference among the animal species. Similarly, the mean residuals of ∑DDTs were also significantly higher in chicken feeds than in pig and cow feeds.

An extensive survey on OCP residuals in eggs, chicken feed and muscle samples was conducted in the 1980s in China before the agricultural pesticides were banned (Pan et al., 1983). Based on 143 chicken feed samples collected from 13 provinces in China, the mean residuals of HCHs were 220 ng g\(^{-1}\), which was significantly higher than what we found in this study (HCH 7.39 ng g\(^{-1}\)); however, the mean residuals of DDTs were 51 ng g\(^{-1}\), which is similar to the results of DDTs (58.23 ng g\(^{-1}\)) measured in the current study. It appears that the HCH residuals in chicken feeds have declined by approximately two orders of magnitude over the past decades, which is consistent with the decrease in HCH residual levels in various bulk environment media because of the ban (Zhu et al., 2005; Chi, 2009; Hu et al., 2009). Based on the study of Yang et al. (2008), dicofol with high impurity of DDT compounds is still widely used in agricultural practice and is an important source of DDT pollution in China; therefore, relatively high DDT residual levels in this study may be attributed to the continuing use of dicofol in agricultural practice, which has resulted in no obvious decrease of DDT residuals in chicken feeds from the 1980s to now. Levels of OCP residuals in chicken feed samples measured in this study were significantly lower than those recently reported for samples gathered from a poultry farm in Punjab, India, where the mean residuals of HCHs and DDTs in chicken feeds were as high as 650 ng g\(^{-1}\) and 910 ng g\(^{-1}\), respectively (Aulakh et al., 2006). Little information about OCP residuals in pig or cow feed samples could be obtained. Additionally, the levels of HCHs and DDTs we found in animal feeds were close to the results obtained in the current study.
of HCHs and DDTs found in farmed Atlantic Salmon aquaculture feeds (Jacobs et al., 2002), but they were lower than the results in fish feed samples reported by Maule et al. (2007).

3.1.2. Compositions of OCP residues in feeds

Industrial HCH mixtures containing 67% α-HCH, 10% β-HCH, 15% γ-HCH and 8% δ-HCH have been used in China (Li et al., 1999). Among the 4 HCH isomers, β-HCH was more persistent than the other isomers in the environment. Fig. 1a shows the mean percentages of HCH isomers in the industrial product and three kinds of animal feeds. Compared to the composition profile of HCHs in the industrial product, the percentage of α-HCH was significantly lower in pig feed (48%) and chicken feed (46%), and the percentage of β-HCH in pig feed and chicken feed was increased to 16% and 36%, respectively (Fig. 1a). However, the percentages of α-HCH and β-HCH in cow feed were 65% and 11%, respectively, which is close to the percentage of α-HCH and β-HCH in the industrial product. The percentage of γ-HCH was higher in both pig feed and cow feed than that in industrial product. Based on the report of Karina et al. (2003), the α-HCH/γ-HCH ratio provides a method of characterizing local lindane (almost pure γ-HCH) release versus global transport of industrial HCH residues. The ratio of α-HCH/γ-HCH in pig feed (2.06) and cow feed (2.67) was lower than that in industrial product (4.47), which suggests that the pollutants of HCHs in both pig feed and cow feed can be attributed to new lindane inputs before the past decade for controlling forest pests as reported by Li et al. (2003), the α-HCH/γ-HCH ratio provides a method of characterizing local lindane (almost pure γ-HCH) release versus global transport of industrial HCH residues. The ratio of α-HCH/γ-HCH in pig feed (2.06) and cow feed (2.67) was lower than that in industrial product (4.47), which suggests that the pollutants of HCHs in both pig feed and cow feed can be attributed to new lindane inputs before the past decade for controlling forest pests as reported by Li et al. (2001). The profile of HCH isomers in pig feed and chicken feed was close to that reported in chicken feed in 1980 (Tao et al., 2009) when HCHs were used extensively in agriculture; however, the HCH ratio of cow feed in this study was close to the industrial product. It’s well known that HCHs have been banned from agricultural use since the early 1980s. On the other hand, it is difficult to accurately detect trace level of OCP residues in bio-samples by GC-ECD, even by GC-MS. Therefore, the composition of HCHs in three kinds of animal feeds might be attributed to some interference from the background of bio-samples.

Industrial DDT is typically composed of 76.1% p,p′-DDT, 20.1% o,p′-DDT, and 3.8% p,p′-DDE (Tao et al., 2009). Fig. 1b shows the mean percentages of DDT isomers and metabolites in technical product and animal feeds. The percentages of p,p′-DDT and o,p′-DDT in pig feed were 44.2% and 5.4%, respectively, which were much lower than those in industrial product. The percentages of p,p′-DDD and p,p′-DDE in pig feed were 10.8% and 39.1%, respectively. In chicken feed, the percentages of p,p′-DDT, p,p′-DDD, and p,p′-DDE were 17.3%, 19.6%, and 25.1%, respectively; however, the percentage of o,p′-DDT in chicken feed was 38.8%, which is higher than that in industrial product. It is well known that o,p′-DDT is 7 times

Table 3

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<td>37.5</td>
<td>nd-9.75</td>
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<td>25.35±21.74</td>
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* Mean and standard deviation values.

b nd = not detectable.

![Fig. 1. Percentage of (a) HCH isomers and (b) DDT isomers and metabolites in technical product and pig, chicken and cow feeds.](image)
The ratio of p,p’-DDE/p,p’-DDT was calculated to be 0.88, 1.45, and 0.16 for pig, chicken, and cow feeds, respectively. Generally, the ratio of DDE/DDT > 1 means the remaining residuals of DDTs are from application in previous decades, whereas a ratio of < 1 suggests more recent application of DDT pesticides. Therefore, the DDT residuals in chicken feed were likely from the earlier applications, while the DDT residuals in manure samples were from the later applications.

The ratio of p,p′-DDT was calculated to be 0.88, 1.45, and 0.16 for pig, chicken, and cow feeds, respectively. Among the 4 DDTs, p,p′-DDT accounted for 39.4%, which indicated that dicofol-type DDT input in the environment resulted in the abnormally high concentrations of OCPs in Dongtai chicken feeds. Besides chicken feed from Dongtai, the concentrations of OCPs in feeds from the same area also exhibited slight differences among three kinds of animals, which might be attributed to the different composition of three

### 3.1.3. Levels of OCPs in animal manures

Table 4 lists the measured HCH and DDT residuals in three kinds of animal manure samples. Over 70% of the manure samples contained two or more HCHs and DDTs in a single sample. Among the 4 HCHs, α-HCH was also detected with the highest occurrence in every kind of animal manures, followed by β-HCH and γ-HCH. Although no δ-HCH was measured in cow feed samples, a considerable occurrence of 25% of cow manure samples contained δ-HCH. The mean concentrations of ∑HCHs were 6.19, 31.46, and 8.86 ng g⁻¹ in pig, chicken, and cow manures, respectively. The mean residuals of ∑HCHs detected in pig and cow manure samples were similar with those measured in pig and cow feed samples, whereas the mean residuals of the 4 HCHs in chicken manure samples were significantly higher than those in chicken feed samples. Similarly, the mean residuals of ∑DDTs were found to be 27.27, 59.43, and 31.33 ng g⁻¹ in pig, chicken, and cow manure samples, respectively, which were also higher than those in the corresponding kind of animal feed samples. Among the 4 DDTs, p,p′-DDE was found in all samples of animal manures, o,p′-DDT and p,p′-DDD were also detected in the manure samples, especially for cows, although o,p′-DDT and p,p′-DDD were not detected in cow feeds (Table 3). The presence of p,p′-DDT in manure usually corresponded to its presence in the animal’s feed. Additionally, the mean concentrations of DDT isomers and metabolites in the same kind of animal manures were found to be in the order of p,p′-DDE > p,p′-DDT > p,p′-DDD > o,p′-DDT, except for p,p′-DDT in chicken manures (Table 4).

The total concentrations of ∑OCPs in feed and manure samples collected from 9 different areas are shown in Fig. 2a and b. On the whole, the concentrations of ∑OCPs in manures were slightly higher than in feeds from the same area, except that the concentrations of ∑OCPs in chicken feeds were much higher than in chicken manures collected from Dongtai as shown in Fig. 2. Such a result showed that residuals of OCPs in feed samples were absorbed and enriched in animal bodies, and then be excreted into animal manures. The concentrations of ∑OCPs in Dongtai chicken feeds were detected to be 406.69 ng g⁻¹, and the total concentrations of p,p′-DDD accounted for 39.4%, which indicated that dicofol-type DDT input in the environment resulted in the abnormally high concentrations of ∑OCPs in Dongtai chicken feeds. Besides chicken feed from Dongtai, the concentrations of ∑OCPs in feeds from the same area also exhibited slight differences among three kinds of animals, which might be attributed to the different composition of three

### Table 4

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*a* Mean and standard deviation values.

*b* nd = not detectable.
animal feeds. On the other hand, the concentrations of ∑OCPs in feed samples had no considerable difference among 9 different area of Jiangsu province except chicken feed from Dongtai, suggesting that there is no new inputs of HCHs and DDTs in Jiangsu province in recent years.

Little information on residuals of HCHs and DDTs in animal manures was available for comparison with the present study; however, the residuals of HCHs and DDTs in sludge have been evaluated in previous studies. Ju et al. (2009) detected OCP compounds in sludge samples in Korea at concentrations of 0.865–3.90 ng g⁻¹ for HCHs and 0.925–16.26 ng g⁻¹ for DDTs, which were lower than residual levels of HCHs and DDTs in this study. The mean residuals of OCPs in this study ranged from 33.46 to 90.89 ng g⁻¹ (Table 4) and were significantly lower than those in sewage sludge samples collected from wastewater treatment plants in China, with mean concentrations of HCHs and DDTs of 19.1 ng g⁻¹ and 229.4 ng g⁻¹, respectively (Wang et al., 2007). In addition, González-López et al. (2005) reported that OCPs were detected at levels ranging between 29 and 392 ng g⁻¹ in stream sediments from an industrial area in 2004. However, levels of HCHs and DDTs in this study were close to, or even higher than, the background value of Phen is higher than other PAH compounds in Nanjing area (Wang et al., 2006b; Yin et al., 2008; Wang et al., 2010), which indicates that the background value of Phen is higher than other PAH compounds in Nanjing area.

If Flt, Pyr, B(a)A, Chry, B(a)P, and D(ah)A in chicken manures are excluded, both the mean concentration of the individual PAHs and the total of ∑PAHs were as follows: cow > chicken > pig. The mean concentrations of the 7 carcinogenic PAHs (7PAHarc) including B(a)A, Chry, B(b)F, B(k)F, B(a)P, D(ah)A and Inpy were 16.82, 16.80, and 79.70 ng g⁻¹ in pig, chicken, and cow manures, respectively. Among the 7PAHarc, Chry, B(b)F, and B(k)F were the individual PAHs with the highest concentrations in three kinds of animal manures, except Chry in chicken manures, and their total concentrations accounted for 69–79% of the 7PAHarc. It is well known that B(a)P is the most carcinogenic of the PAHs. The mean concentration of B(a)P varied from 0.72 ng g⁻¹ in pig manure, to 0.47 ng g⁻¹ in chicken manure, to 1.34 ng g⁻¹ in cow manure, which contributed 1.7–4.3% of the 7PAHarc. The maximum concentration of B(a)P was 2.47 ng g⁻¹ in one cow manure sample, which was much lower than the maximum permissible concentration of 3.0 ng g⁻¹ in sludge for land application in China (GB 18918–2002) (Environmental Protection Administration, 2002). The European Union (EU) proposed that the "sum of PAHs" including Ace, Flu, Phen, Flt, Pyr, B(b)F, B(k)F, B(a)P, B(ghi)P and Inpy should not exceed 6 μg g⁻¹ in sludge for land application (CEC, 2000).

The concentrations of PAHs in manures in Nanjing area were much lower than the permissible value of 6 μg g⁻¹ in sludge for land application (CEC, 2000). Land application of animal manures is a common practice in many parts of the world including China, so the "EU total PAH" concentrations (EU ∑PAHs) are listed in Table 5. The "EU ∑PAHs" in this study ranged from 104.30 to 304.46 ng g⁻¹ in the manures, which were much lower than the permissible value of 6 μg g⁻¹.

Rey-Salgueiro et al. (2008a,b) reported the residual levels of 10 PAHs including B(b)F, B(k)F, B(a)P, B(ghi)P, Inpy, B(a)A, D(ah)A, Chry, dibenz(a)pyrene and benzofluoranthene in four kinds of animal manures. The total concentrations of 10 PAHs were 2.5, 2.6, 4.4, and 9.0 ng g⁻¹ in pig, horse, rabbit, and cow manures, respectively. Dibenz(a)pyrene and benzofluoranthene were not detected in the present study, but the total levels of the other 8 PAHs varied from 17.33 ng g⁻¹ in pig manure, to 19.04 ng g⁻¹ in chicken manure, to 83.14 ng g⁻¹ in cow manure. These values were much higher than the results of total PAHs reported by Rey-Salgueiro et al. (2008a,b). On the other hand, B(a)P measured in this study was at the same levels

### Table 5

<table>
<thead>
<tr>
<th>Substance</th>
<th>Pig (n=4)</th>
<th>Chicken (n=2)</th>
<th>Cow (n=2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td></td>
<td>ng g⁻¹</td>
<td>ng g⁻¹</td>
<td>ng g⁻¹</td>
</tr>
<tr>
<td>Nap</td>
<td>7.21–32.11</td>
<td>14.95 ± 11.78</td>
<td>14.96 ± 31.23</td>
</tr>
<tr>
<td>Ace</td>
<td>nd-1.01</td>
<td>0.25 ± 0.31</td>
<td>1.41 ± 2.00</td>
</tr>
<tr>
<td>Flu</td>
<td>3.28–18.90</td>
<td>8.15 ± 7.30</td>
<td>12.13 ± 16.83</td>
</tr>
<tr>
<td>Phen</td>
<td>39.05–106.00</td>
<td>60.95 ± 31.47</td>
<td>84.46 ± 118.95</td>
</tr>
<tr>
<td>Anth</td>
<td>0.54–1.20</td>
<td>0.92 ± 0.30</td>
<td>1.70 ± 2.40</td>
</tr>
<tr>
<td>Flt</td>
<td>10.62–24.54</td>
<td>16.40 ± 5.86</td>
<td>2.87–16.48</td>
</tr>
<tr>
<td>Pyr</td>
<td>48.1–24.30</td>
<td>10.00 ± 9.54</td>
<td>9.52–30.12</td>
</tr>
<tr>
<td>B(a)A</td>
<td>2.23–2.69</td>
<td>2.49 ± 0.21</td>
<td>3.98–24.72</td>
</tr>
<tr>
<td>Chry</td>
<td>3.90–8.01</td>
<td>5.76 ± 1.71</td>
<td>7.34–38.72</td>
</tr>
<tr>
<td>B(b)F</td>
<td>2.75–8.44</td>
<td>4.63 ± 2.60</td>
<td>3.14–29.34</td>
</tr>
<tr>
<td>B(k)F</td>
<td>1.14–2.03</td>
<td>1.58 ± 0.38</td>
<td>2.37–16.48</td>
</tr>
<tr>
<td>B(a)P</td>
<td>0.28–1.84</td>
<td>0.72 ± 0.75</td>
<td>0.47 ± 0.67</td>
</tr>
<tr>
<td>D(ah)A</td>
<td>0.32–0.78</td>
<td>0.53 ± 0.20</td>
<td>0.91–5.70</td>
</tr>
<tr>
<td>B(ghi)P</td>
<td>nd–0.81</td>
<td>0.51 ± 0.38</td>
<td>1.18–5.73</td>
</tr>
<tr>
<td>Inpy</td>
<td>0.84–1.34</td>
<td>1.11 ± 0.21</td>
<td>1.83–9.40</td>
</tr>
<tr>
<td>∑15PAHs</td>
<td>81.75–231.32</td>
<td>128.94 ± 60.81</td>
<td>142.22 ± 199.84</td>
</tr>
<tr>
<td>∑7PAHarc</td>
<td>13.52–23.56</td>
<td>16.82 ± 4.69</td>
<td>16.80 ± 23.28</td>
</tr>
<tr>
<td>∑EU ∑PAHs</td>
<td>66.63–186.66</td>
<td>104.30 ± 56.16</td>
<td>116.94 ± 164.38</td>
</tr>
</tbody>
</table>

| a | Mean and standard deviation values. |
| b | nd = not detectable. |
| c | ∑7PAHarc: B(a)A, Chry, B(b)F, B(k)F, B(a)P, D(ah)A and Inpy. |
as those reported by Rey-Salgueiro et al. (2008a,b), where the concentration of B(a)P varied from 0.8 ng g$^{-1}$ to 2.5 ng g$^{-1}$ in four kinds of animal manures. No more reported data on residues of PAHs in animal manures were available for comparison with the present study. It is well known that wood ash is usually used as a fertilizer in soils, and some investigations have reported considerable concentrations of PAHs in wood ash (Rey-Salgueiro et al., 2004; García-Falcón et al., 2006; Pérez-Gregorio et al., 2010). The total levels of PAHs in animal manures in this study were quite lower than those found in wood ash samples (Rey-Salgueiro et al., 2004; García-Falcón et al., 2006). The occurrence of PAHs in sludge samples has been investigated since the 1970s in industrial countries, such as the USA, UK and Canada. The mean residuals of 16 PAHs as priority pollutants in the US EPA list were generally from 1 to 100 μg g$^{-1}$, although values up to 2000 μg g$^{-1}$ have also been reported (Perez et al., 2001; Stevens et al., 2003; Durand et al., 2004; Villar et al., 2006; Cai et al., 2007; Dai et al., 2007). Among the 16 PAHs, the total concentrations of the 7 carcinogenic PAHs ranged from 0.016 to 25 μg g$^{-1}$ in sludge samples (Blanchard et al., 2004; Cai et al., 2007; Dai et al., 2007). From the 16 PAHs, the total concentrations of the 7 carcinogenic PAHs ranged from 0.016 to 25 μg g$^{-1}$ in sludge samples (Blanchard et al., 2004; Cai et al., 2007; Dai et al., 2007). The occurrence of PAHs has also been studied in wood ash samples (Rey-Salgueiro et al., 2004; García-Falcón et al., 2006; Khadhar et al., 2010). Both the total levels of 15 PAHs and 7 carcinogenic PAHs in this study were significantly lower than the results of those reported in sludge samples described above.

### 3.2.2. Composition of PAHs residuals in animal manures

The composition pattern of PAHs by ring size in three kinds of animal manures is presented in Fig. 3. PAHs with 3 rings appeared to be the primary components, followed by 4- and 2-ring PAHs. The total levels of 3-ring PAHs varied from 172.32 to 443.61 ng g$^{-1}$ (Fig. 3), and accounted for 56.9–60.7% of the total PAHs. It is reported that PAHs with 4–6 rings have a greater carcinogenic potential than those with 2, 3, or 7 rings. Fig. 3 shows that PAHs with 4 rings were found to be relatively high in pig and cow manures, with concentration levels of 97.83 and 178.73 ng g$^{-1}$, respectively; however, the concentrations of 4-ring PAHs were quite low in chicken manures (26.77 ng g$^{-1}$). Levels of 4-ring PAHs measured in this study were remarkably higher than those found in animal manures collected from farms in Spain, where the concentrations of PAHs with 4 rings ranged from 1.4 in pig manure to 6.5 ng g$^{-1}$ in cow manure (Rey-Salgueiro et al., 2008a,b). PAHs with 2-rings were also at the same concentrations as PAHs with 4-rings and ranged from 74.05 to 129.55 ng g$^{-1}$ (Fig. 3). PAHs with 5–6 rings were much lower than PAHs with 4–5 rings in any kind of animal manures. The mean concentrations of total PAHs with 5–6 rings were 11.04, 11.31, and 27.42 ng g$^{-1}$ in pig, chicken, and cow manures, respectively, and they only contributed 2.14–3.98% of the total PAHs. The levels of PAHs with all numbers of rings in Fig. 3 were in the order of cow > pig > chicken. A similar trend was observed in the investigation of Rey-Salgueiro et al. (2008a,b), and the levels of PAHs in cow manures were higher than those in pig manures. Previous studies reported that the exposure of pigs and chickens to PAHs, concluding that feed was the major source of PAHs for both species (about 99%), whereas the contribution of dermal and inhalation exposure was negligible (Raszyn et al., 1998; Ciganek et al., 2002). Generally, pigs and chickens are permanently housed and fed indoors. Cows are normally provided feed, but they also graze outside every day. This fact could explain the lower PAH levels detected in pigs and chickens. According to Raszyn et al. (1999), atmospheric fall-out of dust particles (on which PAHs are bound) on plants in the vegetative period is considered to be the major contamination source of feeds of plant origin.

### 4. Conclusions

Multiple HCH and DDT isomers and metabolites can be simultaneously detected in most pig, chicken, and cow feed and manure samples collected from 12 feedlots in Jiangsu province, China. With regards to HCHs, α-HCH was the most abundant compound and was present in more than 80% of all kinds of animal feeds and manures. For DDTs, p,p′-DDE and p,p′-DDT were predominant in all feed and manure samples. The mean residuals of OCPs ranged from 25.35 to 65.62 ng g$^{-1}$ in animal feeds, and from 33.46 to 90.89 ng g$^{-1}$ in animal manures. Based on the profiles of HCHs and DDTs, the residuals of indane and DDTS in animal feeds could be attributed to new inputs in the past several years. Of the 15 priority PAHs, Phen was the most abundant PAH species and accounted for approximately 50% of the total PAHs in each kind of animal manure, followed by Nap and Flt. The distribution of PAHs with different rings indicated that PAHs with 3 rings were the primary components in pig, chicken, and cow manures, followed by 4-ring and 2-ring PAHs. The residual levels of OCPs and PAHs in animal manures found in the present study were lower than those reported in sludge samples in China.

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**Fig. 3.** Distribution of PAHs in relation to ring number in pig, chicken and cow manures.

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